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Influence of Two *Desmodium* Species Root Exudates on Haustorium Initiation and Attachment in *Striga hermonthica* (Del.) Benth

Khogali. I. Idris[1], Abadalla M. Hamodun[2], Zeyuar R. Khan[3], Ahmed Hassanali[3] and Abdel Gabar T. Babiker[4]

[1]Agricultural Research Corporation, Shambat Research Station, P. O. Box 30 Khartoum North, Sudan
[2]Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan
[3]International Centre of Insect Physiology and Ecology, Nairobi, Kenya
[4]College of Agricultural Studies, Sudan University of Science and Technology, Khartoum North, Sudan

Abstract: *Striga hermonthica* is a parasitic weed that causes significant yield losses in many important crops and affects the livelihood of millions of people worldwide. A study was undertaken to examine the effects of *Desmodium. uncinatum* (Jacq.) DC and *D. dichotomum* (Klein) DC root exudates on initiation of haustorium and attachment of *S. hermonthica*. A series of laboratory experiments was conducted at the Gezira Research Station, Wad Medani, Sudan. *D. uncinatum* and *D. dichotomum* root exudates, applied 24 hours subsequent to GR 24, a synthetic germination stimulant, or simultaneously with it, did not induce haustorium initiation. *D. uncinatum* and *D. dichotomum* curtailed striga attachment to sorghum roots.

Key words: Striga; *Desmodium* spp.; root exudates; haustorium initiation; attachment

INTRODUCTION

Parasitic weeds develop a strong sink, which allows them to remove water, minerals and photosynthates from the crop. Thus, infection by parasitic weeds reduces the ability of the hosts to grow and yield (Joel *et al*. 2007). Striga (witch weed) causes significant yield and quality losses

in many important crops and affects the livelihood of millions of people worldwide (Parker and Riches 1993). The root parasites, *Striga* spp., exert the greatest damage prior to their emergence (Parker 1991; Matusova *et al*. 2005; Sauerborn *et al*. 2007), and the majority of field losses may occur before diagnosis of infection (Joel 2000). The most destructive *Striga* species on cereal crops are *S. hermonthica* and *S. asiatica.*

About 21 million hectares of cereals in Africa were estimated to be infested by striga causing an annual grain loss of about 8 million tons (Gressel *et al*. 2004). The seeds, which are the main agent of dispersal, have prolonged viability and special germination requirements. To germinate, a striga seed requires a pre-treatment “conditioning" in a moist environment followed by a subsequent exposure to a germination stimulant exuded by the roots of the host and some non-host plant species. Striga germination stimulants isolated from host and non-host species, showed striking structural similarities and are collectively known as sorgolactones (Matusova *et al*. 2005).

The developmental stages of striga comprise a number of mechanisms that ensure close coordination of the parasite life cycle and that of the host (Bouwmeester *et al*. 2003). Therefore, the life cycle of the parasite could be a suitable target for its control. Control measures should focus on reduction of the soil seed bank and on perturbation of the parasites early developmental stages. It is noteworthy that most of the damage caused to the host is attained during the subterranean stages of growth of the parasite (Parker and Riches 1993; Matusova *et al*. 2005).

Seed germination and attachment are key phases in the life cycle of parasitic plants. Thus, the ideal solution to the problem of striga parasitism would be inhibition of attachment of the parasite to the host root without impairing germination. Such a solution results in depletion of seed reserves in the soil in addition to curtailment of damage to the host by the parasite. Most of the available means of control, viz. resistant varieties and cultural, chemical and biological methods, are either not satisfactory, inconsistent in performance, have no effects on the current crop or expensive for subsistence farmers. The need for a management

approach that provides a higher level of protection, does not involve a high level of skill, environmentally friendly, cost effective and sustainable is imperative. Farmers are in need for low-input solutions to the problem. In ICIPE, Khan *et al.* (2000, 2001, 2002, 2006, 2007 and 2008) demonstrated that intercropping maize or sorghum with the fodder leguminous weeds *Desmodium uncinatum* and *D. intortum* (Mill.) Urb. of the family Fabaceae (Leguminosae) significantly reduced *S. hermonthica* infestation and increased grain yield. In Sudan, different *Desmodium* spp. viz. *D. dichotomum*. , *D. adsendens* (SW.) DC. , *D. iasiocarpum* (Beauv.) DC. and *D. repandum* (Vahl) DC were reported, mainly in the rainfed areas (Andrews 1952). This study was, therefore, conducted to examine the effect of two *Desmodium* species root exudates on haustorium initiation and attachment of *S. hermonthica*.

MATERIALS AND METHODS

Plant material

Seeds of *D. uncinatum* were obtained from the International Centre of Insect Physiology and Ecology (ICIPE) and those of D. *dichotomum* were collected from Damazin, Blue Nile State, Sudan. Seeds of the sorghum cultivar Arfa Gadamak and S. *hermonthica* were obtained from the stock of the weed control unit at Wad Medani, Sudan.

S. hermonthica seeds were sterilized in 1% sodium hypochlorite solution (NaOCl) for 5 minutes. NaOCl was drained, seeds were thoroughly washed with distilled sterilized water, air dried and kept in small closed glass vials in a dark cupboard at ambient temperature till used.

Preconditioning of striga seeds

Glass- fibre filtre papers (GFFP), placed in Petri dishes saturated with distilled water, were cut into 5 mm discs and placed in 9 cm Petri dishes, lined with moist glass fibre filtre paper. The sterilized, dried striga seeds were aseptically sprinkled on the discs (approximately 20 to 50 seeds per disc). The filtre papers were then rewetted with distilled water so that the seeds are sufficiently moistened. The seeds were incubated at 30°C in the dark for 10 days prior to germination assays.

Experiment I

Effect of *Desmodium* species root exudates on haustorium initiation in *S. hermonthica:* *Desmodium uncinatum* and *D. dichotomum* seedlings (25 each) were grown in small cups on rock-wool and incubated at 30°C for 10 days. Root exudates were collected, under suction, using a pump. Discs containing pre-conditioned striga seeds were transferred to plastic culture plates with 24 wells. The seeds were treated with 2,6- dimethoxy-p-benzoquinone (DMBQ, a synthetic haustorium inducer) at 10µM.

D. uncinatum and *D. dichotomum* root exudates at 40µl, each alone or in mixture with DMBQ, were added to striga seeds 24 hours subsequent to GR 24 (0.1 ppm) or simultaneously with it. The plates were sealed with parafilm, wrapped with aluminum foil, placed in black polyethylene bags and incubated at 30ºC in the dark for 24 hours. Each *Desmodium* species was tested in a separate experiment. The treatments were arranged in a complete randomized design with four replicates. Striga germilings were examined for haustorium induction 2 days after incubation.

Experiment II

Effects of *Desmodium* species on *S. hermonthica* parasitism: A three days old sorghum (cv. Arfa Gadamak) seedlings together with D. *dichotomum* and *D. uncinatum* seedlings (0, 1, 2, 3 and 4 in number) were transferred to rock-wool, placed in 9 cm plastic Petri dishes with lateral openings to allow emergence of sorghum and *Desmodium* shoots. Conditioned striga seeds, placed on discs of glass fibre papers (0.5 mm), treated with GR 24 at 0.1 ppm or distilled water, were placed near the sorghum roots. The Petri dishes were sealed with parafilm, wrapped with aluminum foil, placed in black polyethylene bags and incubated at 30ºC in continuous light. Sterilized distilled water was added to each Petri dish as needed. Each *Desmodium* species was tested in a separate experiment. The treatments were arranged in a complete randomized design with three replicates. Striga attachment was examined 7 days after transfer.

In both experiments, data on haustorium initiation and attachment percentage were transformed to arcsine and subjected to analysis of variance.

RESULTS AND DISCUSSION

Experiment I

Effect of *Desmodium* species root exudates on haustorium initiation in *S. hermonthica*: DMBQ at 10µM, applied 24 hours subsequent to GR 24 or in mixture with it, induced 47% and 38% haustorium initiation, respectively, in *D. uncinatum* (Table 1). However, simultaneous application of GR 24 and *D. uncinatum* root exudate and DMBQ resulted in 23% haustorium initiation. *D. uncinatum* root exudate applied 24 hours subsequent to GR 24 or simultaneously with it completely inhibited haustorium initiation (Table 1). DMBQ, applied 24 hours subsequent to GR 24 at 0.1 ppm or in mixture with it induced 56% and 86% haustorium initiation, respectively, in *D. dichotomum* (Table 1). DMBQ in mixture with *D. dichotomum* root exudate applied 24 hours subsequent to GR 24 reduced haustorium initiation to 50%. However, simultaneous application of GR 24, *D. dichotomum* root exudate and DMBQ resulted in 44% haustorium initiation (Table 1). *D. dichotomum* root exudate applied 24 hours subsequent to GR 24 or simultaneously with the stimulant inhibited haustorium initiation (Table 1).

D. uncinatum and *D. dichotomum* root exudates applied 24 hours subsequent to or simultaneously with GR 24 did not induce haustoria. Similar findings were reported by Khan *et al*. (2002) who found that chemical components of *D. uncinatum* root exudates give a significant inhibition of haustorial growth. Moreover, Khan *et al*. (2008) reported that root exudates of *D. uncinatum* contain novel flavonoids, some of which stimulate germination of striga and others dramatically inhibit its subsequent development, including radicle growth. Other legumes also produce striga germination stimulants, but demonstrate no significant post-germination allelopathic effects. This suggests close similarity between the two groups of legumes differentiated by a lack of specific tailoring enzymes, e.g. *C*- glycosyl transferase, that converts common precursors to highly active post-germination inhibitors (Pickett *et al*. 2007). Haustorium initiation takes place subsequent to striga seeds germination. As a result, the germinated seed either receives the signal to attach to the host or shrivels and die.

Table 1. Influence of *Desmodium uncinatum* and *D. dichotomum* root exudates on haustorium induction by 2, 6- dimethoxy-p-benzoquinone (DMBQ) in *S. hermonthica*

Treatment	Haustorium initiation percentage	
	D. uncinatum	*D. dichotomum*
GR 24 + DMBQ (a)	(47) 43	(56) 48
GR 24 +DMBQ (b)	(38) 37	(86) 73
GR 24 + RE +DMBQ (c)	(38) 38	(50) 45
GR 24 + RE+ DMBQ (d)	(23) 28	(44) 41
GR 24 + RE (e)	(0) 0.41	(0) 0.41
GR 24 + RE (f)	(0) 0.41	(0) 0.41
SE±	3.90	4.95

a = DMBQ applied 24 hours subsequent to GR 24, b = DMBQ applied simultaneously with GR 24, c = DMBQ in mixture with *Desmodium* root exudates applied 24 hours subsequent to GR 24, d = DMBQ in mixture with *Desmodium* root exudates applied simultaneously with GR 24, e = *Desmodium* root exudates applied 24 hours subsequent to GR 24, f = *Desmodium* root exudates applied simultaneously with GR 24 .Data are arcsine transformed. Figures in parentheses are actual data. Each species was tested in a separate experiment

Allelopathy has been reported to be the cause of the reduction of *S. hermonthica* infection in intercropping with *D. uncinatum*. This practice resulted in inhibiting the development of striga haustoria but not of seed germination (Khan *et al.* 2002).

The allelopathic effect of chemicals exuded from the roots that interfere with haustorial development, combined with the potent chemical stimulants causing suicidal germination, provide not only direct witch weed control, but also a significant depletion of viable seeds in the soil (Khan *et al.* 2002).

Experiment II

Effect of *Desmodium* spp. root exudates on *S. hermonthica* attachment: Striga seeds, conditioned in distilled water and placed near sorghum roots, displayed 6% attachment within 7 days after incubation. Striga germilings, resulting from seeds conditioned in distilled water, treated with GR 24 and placed in the vicinity of sorghum roots, displayed 2% attachment after placement in proximity of sorghum roots (Table 2). In the presence of *D. uncinatum* roots, striga germilings displayed no attachment (Table 2). On the other hand, the germilings resulting from seeds conditioned in water, treated with GR 24 and placed near sorghum roots, displayed 37% attachment, 7 days after incubation. Striga germilings resulting from seeds conditioned in distilled water and placed in the vicinity of sorghum roots displayed 11% attachment (Table 2). The attachment decreased with increasing number of *D. dichotomum* plants. *D. dichotomum* at 1, 2, 3 and 4 plants per Petri dish reduced attachment of striga germilings, induced by GR 24 by 41%, 30%, 43% and 57%, respectively. The germilings resulting from seeds conditioned in water and not treated with GR 24, invariably, showed no attachment to sorghum roots in the presence of *D. dichotomum* (Table 2).

Table 2. Effect of *D. uncinatum* and *D. dichotomum* root exudates on *S. hermonthica* attachment 7days after incubation

Desmodium density	Attachment percentage			
	D. uncinatum		*D. dichotomum*	
	GR 24	Distilled water	GR 24	Distilled water
0	(2) 7	(6) 14	(37) 37	(11) 12
1	(0) 0.41	(0) 0.41	(22) 28	(0) 0.41
2	(0) 0.41	(0) 0.41	(26) 30	(0) 0.41
3	(0) 0.41	(0) 0.41	(21) 27	(0) 0.41
4	(0) 0.41	(0) 0.41	(16) 23	(0) 0.41
SE±	1.13		4.31	

Data are arcsine transformed. Figures in parentheses are actual data. Each species was tested in a separate experiment**.**

The investigation showed that the presence of *D. uncinatum* and *D. dichotomum* reduced attachment of striga to sorghum roots. This finding suggests the presence of haustorium and/ or radicle elongation inhibitors in the root exudates of D. *uncinatum* and *D. dichotomum.* The similar effects of *D. dichotomum* and *D. uncinatum* may indicate comparable phytochemical and physiological attributes.

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تاثير أفرازات جذور نوعان من جنس ابوعريضة (*Desmodium* spp.) علي بدء نشوء عضو الالتصاق و الالتصاق في طفيل البودا *Striga hermonthica* (del.) Benth.

خوجلي عزالدين ادريس[1] و عبدالله محمد حمدون[2] و زهير رحمان خان[3]
و احمد حسن علي[3] وعبدالجبار الطيب بابكر[4]

[1]**هيئة البحوث الزراعية، محطة بحوث شمبات ، الخرطوم بحري- السودان**
[2]**كلية العلوم الزراعية ، جامعة الجزيرة ، واد مدني- السودان**
[3]**المركز الدولي لفسيلوجيا وبيئة الحشرات، نيروبي- كينيا**
[4]**كلية الدراسات الزراعية، جامعة السودان للعلوم والتكنولوجيا، شمبات- السودان**

المستخلص: تعد البودا من الحشائش الطفيلية التي تسبب خسائر معنوية في انتاجية كثير من المحاصيل المهمة وبالتالي تؤثر علي حياة الملايين من البشر. نفذت هذه الدراسة بهدف التعرف علي تاثير افرازات جذور *Desmodium uncinatum* و *D. dichotomum* المعروفه محليا بابوعريضه علي بدء نشوء عضو الالتصاق و الالتصاق في طفيل البودا. أجريت سلسلة من التجارب المعملية بمحطة بحوث الجزيرة، واد مدني، السودان. أظهرت النتائج ان اضافة أفرازات جذور نباتات ابوعريضة بعد 24 ساعة من اضافة ال GR 24 (المحفز لأنبات بذور البودا) او اضافتهما معا في نفس الوقت ادت الي الحد نهائيا من نشوء عضو الالتصاق. كما اوضحت النتائج ان نمو جذور *D. uncinatum* و *D. dichotomum* ادي الي الحد من أو عدم التصاق البودا بجذور الذرة الرفيعة.